Science of the Total Environment 615 (2018) 1438–1445

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Reptile road-kills in Southern Brazil: Composition, hot moments and hotspots

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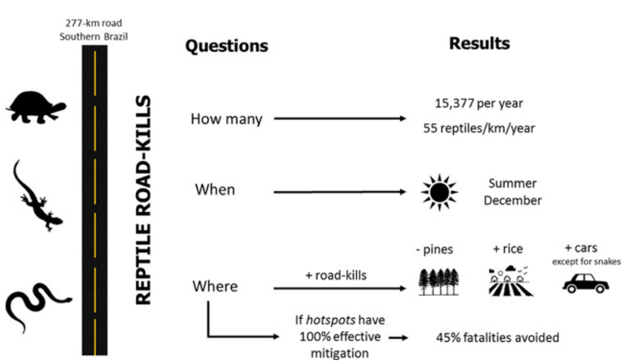
HIGHLIGHTS

- Estimate of 15,377 freshwater turtles, lizards, and snakes road-killed per year
- Road-kill hot moments in summer, especially in December for lizards and snakes
- Road-kill hotspots highly coincident among freshwater turtles, lizards, and snakes
- Positive effects of traffic and rice plantation, and negative of pine plantation
- Hotspots (21% of the road extent) included 45% of reptile fatalities.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 June 2017
Received in revised form 2 September 2017
Accepted 6 September 2017
Available online 16 October 2017

Editor: Henner Hollert

Keywords:
Carcass detection
Carcass removal
Mitigation
Road ecology
Road-kill aggregation
Wildlife-vehicle collisions

ABSTRACT

Understanding road-kill patterns is the first step to assess the potential effects of road mortality on wildlife populations, as well as to define the need for mitigation and support its planning. Reptiles are one of the vertebrate groups most affected by roads through vehicle collisions, both because they are intentionally killed by drivers, and due to their biological needs, such as thermoregulation, which make them more prone to collisions. We conducted monthly road surveys (33 months), searching for carcasses of freshwater turtles, lizards, and snakes on a 277-km stretch of BR-101 road in Southernmost Brazil to estimate road-kill composition and magnitude and to describe the main periods and locations of road-kills. We modeled the distribution of road-kills in space according to land cover classes and local traffic volume. Considering the detection capacity of our method and carcass persistence probability, we estimated that 15,377 reptiles are road-killed per year (55 reptiles/km/year). Road-kills, especially lizards and snakes, were concentrated during summer, probably due to their higher activity in this period. Road-kill hotspots were coincident among freshwater turtles, lizards, and snakes. Road-kill distribution was negatively related to pine plantations, and positively related to rice plantations and traffic volume. A cost-benefit analysis highlighted that if mitigation measures were installed at road-kill hotspots, which correspond to 21% of the road, they could have avoided up to 45% of recorded reptile fatalities, assuming a 100%

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https://doi.org/10.1016/j.scitotenv.2017.09.053

mitigation effectiveness. Given the congruent patterns found for all three taxa, the same mitigation measures could be used to minimize the impacts of collision on local herpetofauna.

1. Introduction

Among several road impacts on wildlife, such as habitat loss, degradation, and fragmentation, fatalities due to vehicle collisions are one of the most concerning impacts (Forman et al., 2003; van der Ree et al., 2015b). Road mortality can cause faster population declines compared to other impacts, such as connectivity reduction (Jackson and Fahrig, 2011; Jaeger and Fahrig, 2004). Road-killing can also foster evolutive changes in populations (Brady and Richardson, 2017). Understanding patterns and processes related to wildlife-vehicle collisions is fundamental for guiding policies to minimize their impact.

Despite the long interest in understanding the effects of reptile-vehicle collisions (Fitch, 1949) and the fact that this group seems to be more affected than other vertebrates (D'Amico et al., 2015; Jochimsen et al., 2014), reptiles are still underrepresented in road ecology literature (Fahrig and Rytwinski, 2009; Gunson et al., 2011). The lesser interest on reptile fatalities is probably explained by the importance that other vertebrate groups, such as medium and large mammals, pose to human safety (Danks and Porter, 2010; Huijser et al., 2009). However, when mitigations are planned aiming to reduce the anthropogenic impact on biodiversity, relying on information available for a single taxonomic group might be ineffective, since road-kill patterns in space (e.g. Teixeira et al., 2013b) and time can vary among distinct taxonomic or functional groups.

Even without knowledge of the demography of local populations, understanding which species and how many animals die on roads can be the first step to assess the potential effects of road mortality on wildlife populations, as well as to define the need for mitigation and support its planning. Road-kill estimates need to incorporate inherent errors of carcass surveys, such as imperfect detection and carcass persistence (Santos et al., 2011; Teixeira et al., 2013a). Studies accounting for these errors on reptile road-kill estimates are rare (but see Gerow et al., 2010 and Teixeira et al., 2013a) and the real magnitude of reptile mortality is certainly underestimated. Road mortality effects are not equally distributed in time and space (Beaudry et al., 2010), therefore assessing road-kill hot moments and hotspots, i.e. periods and locations with significantly higher fatalities, is important to propose periods and locations for mitigation (Gunson and Teixeira, 2015).

Both intrinsic and extrinsic factors can affect road-kill distribution. Species have life traits that make them more vulnerable to vehicle collisions, such as mobility and behavioral responses to traffic volume (Jacobson et al., 2016; Lima et al., 2014). Landscape and road characteristics certainly affect road-kill patterns. Variables related to the presence and distance of water bodies and to traffic volume are recognized as important factors determining spatial patterns of reptile fatalities on roads (Glista et al., 2008; Langen et al., 2012, 2009).

Knowledge of environmental or road attributes related to higher road-kill probability can be used to identify priority periods and locations for mitigation on other roads, for which road-kill data are unavailable (D'Amico et al., 2015; Glista et al., 2008). Although there is a number of possible mitigation strategies potentially beneficial for reptiles, passages associated with funneling fencing seem to be the most effective for multispecies purposes (Jackson et al., 2015). Currently available technologies allow for implementation of mitigation structures during road operation with relatively little trouble to traffic, whereas proper design, implementation and maintenance could result on nearly absolute effectiveness (Aresco, 2005; Van der Ree and Tonjes, 2015).

In this study, we described road-kill patterns for freshwater turtles, lizards, and snakes on BR-101 road, Southernmost Brazil. We evaluated which species are road-killed, how many reptiles are killed on this road (considering carcass removal and detection based on experiments), and when and where these road-kills are concentrated. We also assessed the relationship of reptile fatalities with land cover and local traffic volume. We expected temporal patterns to show a concentration of fatalities in summer months due to higher reptile activity in this period. We expected distinct spatial distribution of fatalities for each group, considering they vary in natural history: freshwater turtles are more associated with aquatic environments, while lizards tend to occupy open and forested areas. For snakes, we expected the spatial pattern of fatalities to be less aggregated due to their higher regional species richness. We also expected that traffic volume would have a stronger association with freshwater turtle fatalities than with other groups because they are less mobile and present a 'pauser' behavior in reaction to upcoming vehicles (Jacobson et al., 2016).

2. Methods

2.1. Study area

We conducted this study on a 277-km stretch of BR-101 road, located at the lowlands of Rio Grande do Sul state, Brazil (initial coordinates 30°9′1.20″S and 50°30′49.33″W, and final coordinates 32°0′23.64″S and 52°2′17.73″W; Appendix A). BR-101 is located in the eastern side of Patos Lagoon, adjacent to the Lagoa do Peixe National Park, a recognized Ramsar site (Ramsar Convention, 1962).This stretch is a two-lane paved road with 11 m of width, a speed limit of 80 km/h, and an average daily traffic (ADT) between 690 and 2900 vehicles, depending on the locality.

2.2. Data collection

We conducted monthly surveys from September 2012 to August 2014, and from February to October in 2015, totaling 33 surveys. Two observers (including the driver) conducted surveys by car at 40–50 km/h (speed limit followed minimum allowed speed according to Brazilian regulation) from dawn to dusk. Detected carcasses were identified to the lowest taxonomic level and their locations were georeferenced with a handheld GPS.

We used vehicle counters (Vehicle Counter Generation III—TRAFx Research Ltd.) to calculate the average daily traffic (ADT) in three locations linking the main regional settlements: Capivari do Sul ($n = 48$ days), Mostardas ($n = 273$ days), and São José do Norte ($n = 498$ days). Since we found a north-to-south decrease in traffic volume, we extrapolated traffic volume for each 2-km road segment by performing a linear regression with the recorded ADT in each surveyed location and the distance to the northernmost city (Capivari do Sul).

We used a land cover map from LANDSAT 5 TM images classification for 2009 (UFRGS-IB-Centro de Ecologia, 2016) with eight classes: wetlands, native forest, dry grassland, water, pine plantation, rice plantation, urban areas and mixed areas (various crops, annual or perennial, and degraded grasslands). We calculated the area of each of the eight land cover classes within a 2-km buffer centered on each 2-km road segment.

2.3. Data analyses

We assumed that collision risk is related to movement capacity, and grouped reptile species according to their behavioral responses to traffic volume for subsequent analyses (Jacobson et al., 2016). Freshwater turtles usually freeze on the road in response to vehicle presence ('pausers'), lizards usually flee ('speeders') and snakes usually do not respond to vehicles ('non-responders') or show responses as 'pausers' or 'speeders' (Jacobson et al., 2016). Therefore, we analyzed freshwater turtles, lizards, and snakes (we included amphisbaenians in snakes group) as separate groups.

2.3.1. Estimates of road-kill magnitude

We estimated road-kill magnitude for all reptiles, freshwater turtles, lizards, and snakes through *Nestimate* function based on Korner-Nievergelt et al. (2011) using the *Carcass* package (Korner-Nievergelt et al., 2015) in R environment (R Core Team, 2016). This estimate considers the detection capacity of the method, carcass persistence on the road, number of surveys and survey interval. This method assumes that search intervals are regular and persistence probability and search efficiency are constant over time. Carcass persistence was estimated based on the exponential model (Korner-Nievergelt et al., 2011).

To assess carcass detection and persistence we performed experiments by placing 56 carcasses of reptiles (five freshwater turtles and 51 snakes) previously collected on BR-101 road on a 30-km stretch of the same road. Detection was evaluated for six survey teams (each of them with two observers following the same method used in regular surveys) who monitored this 30-km stretch without knowing the location of carcasses. After all teams had surveyed the road, we checked every carcass placed and found that ten had been removed. Therefore, we considered 46 carcasses (four freshwater turtles and 42 snakes) for evaluating the probability of detection of the method: 14 carcasses smaller than 15 cm, 27 from 15 to 35 cm, and five larger than 35 cm. To estimate carcass persistence, we used the total 56 carcasses, checking their persistence for five consecutive days. We used *search.efficiency* and *persistence.prob* functions from the *Carcass* package to calculate carcass detection and persistence separately for freshwater turtles and snakes. As we did not include lizard carcasses in our experiment, we used detection and persistence values from snake carcasses. We used carcass detection and persistence calculated considering all carcasses as values for reptiles.

2.3.2. Road-kill hot moments

We analyzed road-kill hot moments for freshwater turtles, lizards, and snakes using circular statistics in Oriana 4.02 software (Kovach, 2004) considering only data collected during the first two continuous years of surveys. Months were converted in angles (30-degree intervals) and the sum of road-kills in each month was used as a frequency for each angle. We then obtained the mean angle, which represents the average period with the highest number of fatalities within the whole period. We assessed the significance of the average period in relation to a uniform distribution of road-kills through the Rayleigh test of uniformity, Z (Kovach, 2004). Then, we calculated the intensity of road-kill concentration through the average period length (r), which varies from 0 (uniform dispersion) to 1 (road-kill concentration in the same direction).

2.3.3. Road-kill hotspots

We evaluated on which scales road-kill hotspots occurred for freshwater turtles, lizards, and snakes using Ripley's K statistic (Levine, 2000; Ripley, 1981) at a bidimensional space in Siriema v.2.0 software (Coelho et al., 2014). We used an initial radius of 300 m, a radius increase of 500 m, and 100 simulations of random distribution events to evaluate clustering significance (99% confidence interval). After the identification of the scales with road-kill hotspots, we performed a 2D HotSpot Identification analysis for recognizing where hotspots were located. We used a 1-km radius and divided the road into 138 segments of 2 km each. We chose this segment length because Ripley's K statistic identified the occurrence of clustering on that scale and because some mitigation measures for reptiles can be easily implemented targeting a 2-km road segment, as for example, a wildlife passage connected by funnel fencing (Baxter-Gilbert et al., 2015). We performed 1000 simulations of random distribution to assess significance of hotspots locations (95% confidence interval). We considered as hotspots all segments with a road-kill intensity value higher than the upper confidence limit (Coelho et al., 2014).

As many hotspots might be identified on a road and, in most cases, there are budget restrictions to mitigating all of them, we evaluated the relative contribution of mitigating hotspots. We calculated the potential reduction in road-kills in the case of mitigating each of the road segments identified as hotspots for at least one of the three reptile groups studied. We sorted hotspots by their intensity and we built a cumulative curve of the number of road-kills recorded at each hotspot location as a proxy of the potential gain obtained by mitigation.

2.3.4. Road-kill association with land cover and traffic volume

To assess the relationship of road-kills of freshwater turtles, lizards and snakes with land cover classes and traffic volume, we fit generalized linear models with a Poisson distribution. We divided the road into 2-km segments, using the same segments from the 2D HotSpot Identification analyses, and the number of road-kills in each segment was used as response variable. Predictive variables were standardized to have a mean of 0 and standard deviation equal to 1.

We used hierarchical partitioning (Mac Nally, 2002) to assess the influence of each predictive variable on the number of road-killed freshwater turtles, lizards, and snakes. Hierarchical partitioning uses models with all combinations of predictive variables to evaluate the independent (I) and joint (J) effect of each of them on the response variable. We tested the statistical significance of the contributions of independent variables using a randomization process (999 randomizations) based on a 95% upper confidence limit (Z-score > 1,96). This statistical analysis was conducted in R (R Core Team, 2016) with the *hier.part* package using log-likelihood as the goodness-of-fit measure (Walsh and Mac Nally, 2013). Then, we assessed the explained deviance of each full model (for freshwater turtles, lizards, and snakes) calculated as 1 − (residual deviance/total deviance).

3. Results

3.1.1. Estimates of road-kill magnitude

We recorded 1353 carcasses of reptiles on BR-101 road, 18% of which were freshwater turtles belonging to four species, 11% were lizards (Argentine Black and White Tegus, *Salvator merianae*), and 70% were snakes belonging to 24 species and one amphisbaenian species (*Amphisbaena trachura*) (Table 1; Appendix B). We estimated carcass detection as 55% (95% IC [26%, 82%]) for freshwater turtles and 23% (95% IC [15%, 34%]) for snakes and lizards. Carcass persistence probability for freshwater turtles was 0.85 in one day (95% IC [59%, 94%]) with a mean persistence time of six days. Carcass persistence probability for snakes was 0.82 in one day (95% IC [76%, 87%]) with a mean persistence time of 5.2 days. After correcting for carcass detection and removal, we estimated a total of 42,287 road-killed reptiles during 33 months of survey (Table 1), which corresponds to 15,377 road-killed reptiles per year (789 freshwater turtles, 1600 lizards, and 10,206 snakes).

3.2. Road-kill hot moments

Fatalities of freshwater turtles were concentrated in January, while fatalities of lizards and snakes were significantly concentrated in December (Fig. 1). Freshwater turtles were the group with the lowest road-kill concentration in time ($r = 0.28$; $Z = 9.7$; $p < 0.001$), followed by snakes with intermediate concentration values ($r = 0.47$; $Z = 176.7$; $p < 0.001$), and lizards with the highest values ($r = 0.80$; $Z = 88.2$; $p < 0.001$).

3.3. Road-kill hotspots

We found road-kill clustering from 300-m to 70-km scales for freshwater turtles, from 300-m to 162-km scales for lizards, and from 300-m

Table 1
Number of observed carcasses and estimates of road-kill magnitude for freshwater turtles, lizards, and snakes during 33 months of surveys on BR-101 road. Lower and upper 95% confidence limits are in parentheses.

Groups	Observed carcasses	Estimates of road-kill magnitude	Estimates of magnitude per year	Estimates of magnitude per km per year
Freshwater turtles	245	2170 (550–9206)	789 (200–3347)	2.8 (0.7–12.1)
Lizards	151	4400 (2545–7671)	1600 (925–2768)	5.8 (3.3–10.8)
Snakes	957	28,069 (16,654–48,631)	10,206 (6056–17,684)	36.9 (21.8–63.8)
TOTAL	1353	42,287 (24,080–73,890)	15,377 (8756–26,869)	55.55 (31.6–97.1)

to 78-km scales for snakes (Appendix C). 2D HotSpot Identification analyses indicated that most hotspots were concentrated in the Northern portion of the road, the segment with the highest traffic volume (Fig. 2). For snakes, we also identified some hotspots also in the Southern part of the road (Fig. 2).

When assessing road segments that were identified as hotspots for at least one of the groups, and assuming the proposed mitigation would be 100% effective, we can infer that 45% of reptile deaths could be avoided if 21.7% of the segments of the road (the 30 hotspot segments) had been mitigated (Fig. 3). That means a twofold efficiency in a cost-benefit relationship (km mitigated/road-kills avoided). If we detail this potential reduction by group, we would reach a 40% road-kill decrease for snakes, 53% for freshwater turtles and 60% for lizards (Fig. 3), a 2–3 cost-benefit rate.

3.4. Road-kill association with land cover and traffic volume

The amount of pine and rice plantations were the most important variables for determining the fatalities of freshwater turtles, lizards, and snakes (Table 2). Road-kills of freshwater turtles showed a significant positive relationship with rice plantations (I% = 26.23), urban areas (I% = 11.77), and traffic volume (I% = 8.68), and a significant negative relationship with pine plantations (I% = 34.07) and dry grasslands (I% = 8.95). For lizards, we found a positive relationship with rice plantations (I% = 33.06) and traffic volume (I% = 20.71), and a negative relationship with pine plantations (I% = 22.74). Snake fatalities were positively related to rice plantations (I% = 22.7) and mixed areas (I% = 8.59), and negatively related to rice plantations (I% = 45,75) and traffic volume (I% = 8.59).

4. Discussion

We estimated that 55.55 reptiles are road-killed per kilometer per year (range: 31–97 reptiles/km/year), totaling >15 thousand road-killed animals every year on a 277-km segment of BR-101 road. The estimated road mortality magnitude obtained in this study corresponds to 30 times the number of observed carcasses during the road surveys, and exceeded the estimates of reptile road-kills on other roads, which did not consider carcasses detection and removal (e.g. de Souza et al., 2015; Hartmann et al., 2011; Pragatheesh and Rajvanshi, 2013). In a study conducted in the Brazilian Pantanal, 58 vertebrate road-kills were recorded per kilometer per year (de Souza et al., 2015), in which mammals were the most representative group (61%) and reptiles corresponded only to 13% of the total. This low reptile representation was also present in other studies (e.g. Bager and Fontoura, 2013). However, it is important to point out that both the relative frequencies and the road-kill rates are underestimated, since carcass detectability is associated with body size (Teixeira et al., 2013a) and lower for reptiles when compared to mammals.

A high proportion of the reptile species known for the region is potentially affected by road-kill. We recorded 72% of the known reptile species pool from the entire coastal lowlands of Rio Grande do Sul (Borges-Martins et al., 2007) and other seven species that were not in their inventory (two freshwater turtles and five snakes). The higher number of snake species recorded as road-kill is in agreement with its higher richness for the region.

The occurrence of a large number of reptile road-kills depends on two factors: (1) availability (higher exposure) and (2) lethality (higher risk of running over). In the case of reptiles, higher exposure to roads may be explained by: higher abundance near roads, necrophagy, and the habit of thermoregulation. The abundance of individuals in habitats along road verges is the most important factor determining availability, but it is rarely estimated to allow comparison (Meek, 2015, 2009). Still, several species of lizards and snakes are active foragers that prefer open environments, increasing the chance of using roads or open vegetation adjacent to roads for foraging (Brehme et al., 2013; Meek, 2009) and turtle females may use road edges for nesting (Aresco, 2004; Dorland et al., 2014). Necrophagy is part of the dietary habit of the only species of lizard recorded (11% of all records) in our study (Kiefer and Sazima, 2002; Sazima and D'Angelo, 2013), and it has been documented for our most recorded snake species (*Philodryas patagoniensis*) as well (Ucha and Santos, 2017). When animals are attracted to roads to feed on road-kills, they expose themselves to the risk of collisions with vehicles. In addition, reptiles might increase their exposure when they thermoregulate, as it has been demonstrated that the asphalt temperature is strongly related to the presence of snakes from different species on roads (Mccardle and Fontenot, 2016).

The second factor determining higher road-kill rates is lethality, which is related to traffic volume and, for the same traffic volume, to drivers' and animals' behaviors, as well as animal size and mobility. Road-kill rates are usually related to high or medium traffic volumes (Gunson et al., 2011), especially for species that do not avoid roads.

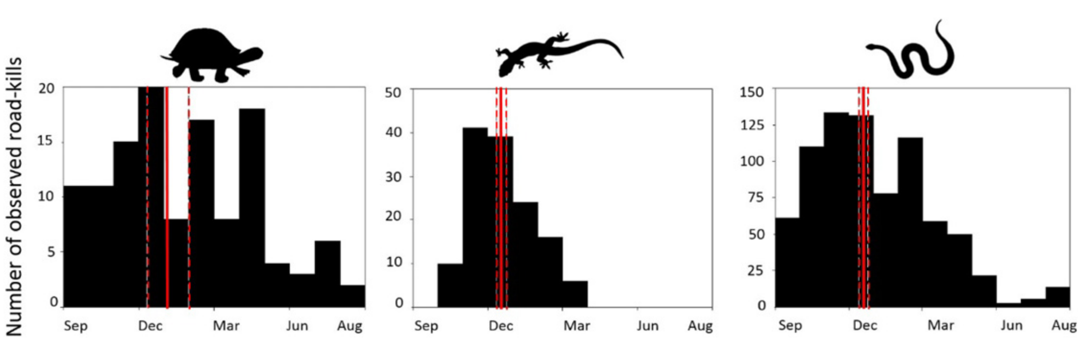

Fig. 1. Road-kill hot moments for freshwater turtles, lizards, and snakes during two years of surveys (September 2012 to August 2014). Mean concentration period (full red lines) and standard error (dashed red lines).

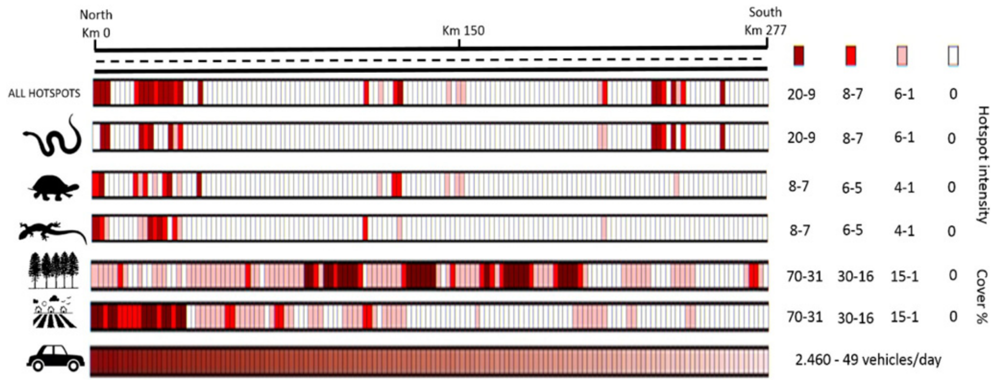

Fig. 2. Spatial distribution of hotspots (all reptile hotspots; snakes, freshwater turtles, and lizards hotspots separately), percentage of pine plantations, rice plantations, and traffic volume (ADT) along BR-101 road. Hotspot values correspond to road-kill intensity values from 2D Hotspot Identification analyses. Each segment corresponds to the 2-km road stretch used as sampling unit in the models.

Several studies demonstrated that drivers' intentional collision with snakes and turtles is higher than observed for control objects (Ashley et al., 2007; Beckmann and Shine, 2012; Langley et al., 1989; Secco et al., 2014), and higher for snakes than for freshwater turtles (Ashley et al., 2007). Crawford and Andrews (2016) demonstrated that drivers would be less upset (a proxy of intention or care by the authors' point of view) when they run over a snake than when they run over a turtle or a large mammal. Considering animal size, Whitaker and Shine (2000) suggested that snakes are a larger target for vehicle collisions because their body is longer in relation to other animals' when they cross the road perpendicularly. In relation to animal mobility, Andrews and Gibbons (2005) pointed out that some snakes and turtles have an immobilization behavior in response to vehicle approximation ('pausers' category in Jacobson et al., 2016) and could be an additional explanation for their higher road-kill rate together with their lower crossing speed.

Road-kill hot moments were concentrated mostly in summer months. This temporal pattern has been demonstrated in other studies for reptiles as a whole (Garriga et al., 2017), for freshwater turtles (Cureton and Deaton, 2012), and for lizards (Meek, 2014). Hot moments have been shown to be related to climate variables, such as temperature and precipitation (Garriga et al., 2017), which influence the breeding season (Cureton and Deaton, 2012), foraging (Meek, 2014), and species movement (Andrews and Gibbons, 2005; Shine et al., 2004). Lizard from the only species recorded in our study (*Salvator merianae*) are inactive almost half of the year (Borges-Martins et al., 2007), with the active period in the warm months, when they move to reproduce and to forage, increasing the probability of using roads. Temporal patterns of fatalities could differ among species (Mccardle and Fontenot, 2016; Meek, 2014), as well as among sex and age classes (Jochimsen et al., 2014), thus temporal patterns could be still more restrictive than observed because it is related to specific behavior characteristics, as thermal biology (Mccardle and Fontenot, 2016).

Road-kill hot moments can be used to plan the implementation of temporary structures such as directional fences, associated with specific wildlife underpasses for reptiles (Baxter-Gilbert et al., 2015; Markle et al., 2017). The implementation of temporal directional fences can be interesting due to their lower costs, even considering the costs of installation and maintenance. Whenever reptiles are the target group for mitigation, the existence of road-kill hot moments allows the concentration of field efforts to evaluate and monitor reptile road-kills during the period with higher fatality frequency. This would abbreviate decision-making and reduce associated costs.

Road-kill hotspots were predominantly coincident between freshwater turtles, lizards, and snakes, except for some aggregations of snake road-kills that were located in the southern portion of the road. The coincidence among hotspots for different groups allows mitigation strategies to be designed for the reptile group as a whole, always considering that mitigation measures must be effective in their

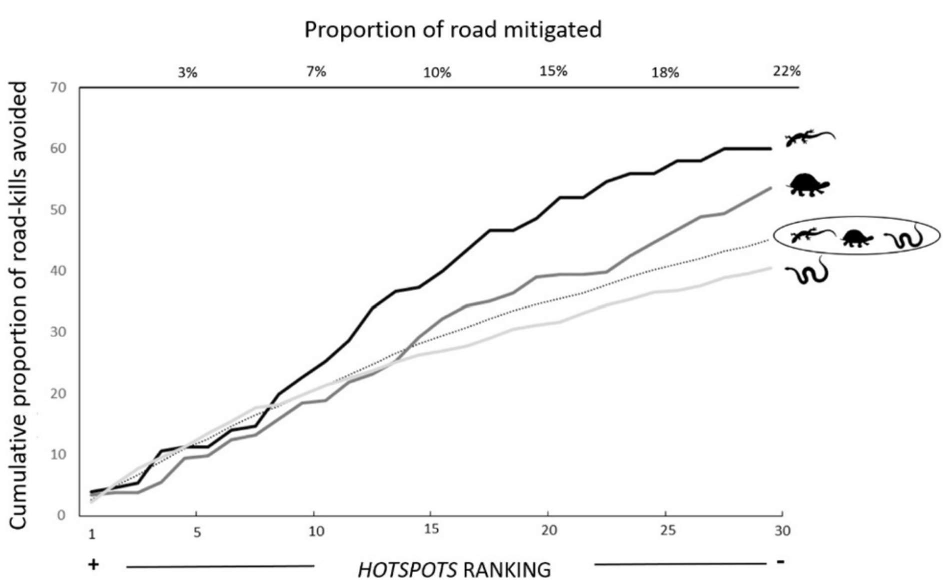

Fig. 3. Cumulative proportion of road-kills avoided for lizards, freshwater turtles, reptiles, and snakes considering the implementation of 100% effective mitigation measures on hotspots locations. Hotspots illustrated correspond to 21.7% of the road (upper x axis). Hotspots were ranked in the lower x axis in decreasing order of aggregation intensity.

Table 2
Results of variables' associations from the hierarchical partition for each group. 'Dev' is the percentage of explained deviance for models including all variables in each reptile group. The sign of each variable is obtained from a Poisson regression model and shows the relationship between each predictive variable and the response variable. 'I' and 'J' are respectively the independent and joint contribution of each variable for each reptile group. 'Total' is the sum of 'I' and 'J'. '%I' is the relative percentage of independent contribution for each variable. 'Z score' is the randomization test of the independent contributions for each predictive variable (* identifies significant variables).

	Freshwater turtles						Lizards						Snakes					
	Dev 39%	I	J	Total	%I	Z.score	Dev 44%	I	J	Total	%I	Z.score	Dev 44%	I	J	Total	%I	Z.score
Traffic volume	+	5.7	7.35	13.1	8.68	2.38*	+	12.9	20.1	33	20.7	6.6*	−	9.84	−8.16	1.68	8.95	3.14*
Water	+	0.33	−0.31	0.02	0.5	−0.53	−	1	0.62	1.62	1.61	−0.16	−	5.03	2.18	7.21	4.58	1.19
Urban area	+	7.73	5.09	12.8	11.8	3.84*	+	4.07	3.58	7.65	6.53	1.5	−	1.41	−1.13	0.28	1.28	−0.16
Wetland	−	0.52	−0.45	0.07	0.78	−0.46	−	1.82	3.44	5.26	2.92	0.33	+	1.86	0.75	2.61	1.69	−0.04
Native forest	+	2.52	−2.47	0.04	3.83	0.71	−	2.21	4.89	7.1	3.54	0.48	+	4.57	2.39	6.96	4.16	1.04
Dry grassland	−	5.88	2.14	8.01	8.95	2.49*	−	2.59	3.78	6.37	4.16	0.79	+	2.54	0.54	3.08	2.31	0.23
Mixed area	+	3.41	−2.38	1.03	5.2	1.28	+	2.95	−2.12	0.82	4.73	0.92	+	9.44	−2.36	7.08	8.59	2.94*
Rice plantation	+	17.2	14.6	31.8	26.2	8.58*	+	20.6	23	43.6	33.1	11.47*	+	25	−6.09	18.9	22.7	9.01*
Pine plantation	−	22.4	10.5	32.9	34.1	11.95*	−	14.2	8.54	22.7	22.7	8.28*	−	50.3	27.2	77.5	45.8	21.12*

implementation and operation, since small installation or maintenance failures can have great consequences on their effectiveness (Baxter-Gilbert et al., 2015). We hereby demonstrated that if effective mitigation measures were installed on top ranking hotspots, which represent a relatively small proportion of the road (21%), they could contribute to reduce observed fatalities in 45%. This twofold cost-benefit ratio was obtained assuming an absolute effectiveness of mitigation that could be attained with well-planned passages associated to drift fences with recurrent maintenance (Jackson et al., 2015; Van der Ree and Tonjes, 2015).

Under some circumstances, such as older roads, the use of hotspots for defining mitigation locations may not be the most adequate measure, as hotspots can change over time (as from high-traffic segments to low-traffic segments), as a consequence of population depletion by road mortality (Teixeira et al., 2017). However, this may not be the case for the road segment studied here, as its paving started in 1993 for the first 120 km (near Mostardas city) and finished in 2009 for the entire 277-km segment (near São José do Norte city). Furthermore, we observed a positive relationship between fatalities and traffic volume (which decreases from north to south), except for snakes, the group with hotspots both at the northern and at the southern portions of the road.

The proportion of pine plantations was the most important land cover variable that influenced fatalities of freshwater turtles, lizards, and snakes, with a relatively strong negative effect. This negative relationship has already been highlighted for other wildlife groups, such as owls (Gomes et al., 2009). Moreover, the impoverishment of habitat and wildlife caused by exotic pine and/or eucalyptus plantations has been extensively documented, especially in grassland dominated landscapes (Berthrong et al., 2009; Brockerhoff et al., 2008; Corley et al., 2006). Even in forest environments, such as in the northeastern Brazilian Amazon, the richness of both amphibians and lizards was lower in eucalyptus plantations than in native primary and secondary forests (Gardner et al., 2007; Saccol et al., 2017). The presence of pine plantations is probably reducing the abundance and richness of reptiles in the areas surrounding the road, decreasing their availability to be road-killed.

Rice plantations coverage was already recognized as determinant for reptile hotspots (Grilo et al., 2016; Seo et al., 2015), as these human-modified environments provide refuge for some wetland species. Water bodies or wetlands at road margins are recognized as important features for determining road-kill aggregations, both for vertebrates in general (Freitas and Federal, 2015) and for freshwater turtles (Cureton and Deaton, 2012; Langen et al., 2012). However, we did not find a relationship between the percentage of water cover and fatalities, probably because the water category considered in our mapping represents only large water bodies (lakes and lagoons) and what probably matters to these animals are the wet areas close to the road, such as puddles and ditches. Not only the cover percentage, but also the distance to water bodies is important for turtles and can show a negative relationship with the presence of freshwater turtle hotspots (Langen et al., 2012). As expected, we found a negative relationship between dry grassland percentage and freshwater turtle fatalities.

For vertebrates in general (Seo et al., 2015), and especially for reptiles, traffic volume is widely recognized as responsible for fatality locations (see Cureton and Deaton, 2012, and Langen et al., 2012 for freshwater turtles). Contrary to this pattern, we found a negative relationship between traffic volume and road-kill density for snakes. Some snakes may be avoiding to cross the road in areas where traffic volume is high (Siers et al., 2016) or some snake populations have already suffered a decline in these areas and have lower abundance, decreasing their interaction with the road (Teixeira et al., 2017). In addition, habitat quality in areas with lower traffic may be better (Shepard et al., 2008), allowing larger populations to thrive.

Regardless of the explanations for the occurrence of road-kills (single or multiple causes or even their interactions), road mortality for the different groups evaluated showed congruent spatial and temporal patterns. Considering this scenario, the best strategy for an effective multispecific mitigation is to diminish the interaction between animals and the road or traffic, providing opportunities for safe crossings at each hotspot segment, associating multiple wildlife passages with directional fences specific for reptiles (Andrews et al., 2015; Jacobson et al., 2016; Markle et al., 2017). To reduce deterioration or even theft of fences, they could be installed temporarily only during summer months, although the cost-benefit of recurrent installation needs to be evaluated in comparison to permanent fences. Absolute exclusion of animals from the road should be followed by frequent maintenance inspections. Also, sufficient jump-out opportunities for animals that get stuck between fences should be provided as a complementary measure (van der Ree et al., 2015a). By adopting this set of relatively low-cost measures, we expect a significant reduction of the present-day carnage observed on this road.

5. Conclusion

Reptile fatalities in the Southern portion of BR-101 road were temporally and spatially aggregated, with hotspots and hot moments overlapping among different reptile groups. The high number of fatalities may be associated with the recent paving of this road (ended in 2009), which influenced traffic volume and vehicle speed. Since then, traffic volume has been rising and is predicted to further increase following higher human occupation in the region. When sorting hotspots by intensity of road-kill hotspots, we demonstrated a cost-benefit rate of mitigation (km mitigated/road-kills avoided) of 1:2 for reptiles and even larger for single groups. By showing that areas of pine and rice plantations and that traffic volume were important for explaining reptile road-kills, we provided some clues for mitigation planning on roads in similar landscapes where road-kill data is not available, and indicated important variables for the development of predictive models.

Acknowledgements

We are grateful to Fernando Ascensão, Maria João Ramos Pereira, Murilo Guimarães, and Nicole da Rosa for all their comments on the manuscript. To Taís de Fátima Guimarães and Bruna Arbo Meneses for helping with land cover data. To all colleagues that collaborated during field surveys. LOG would like to thank CAPES for her scholarship (process n. 88881.132536/2016-01) FZT received a PNPD/CAPES fellowship. VAGB received support from the TULIP Laboratory of Excellence (ANR-10-LABX-41 and 394 ANR-11-IDEX-002-02) and from a Region Midi-Pyrenees project (CNRS 121090).

Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.scitotenv.2017.09.053. These data include the Google map of the surveyed road with hotspots intensity of freshwater turtles, lizards and snakes.

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